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The Protective Role of Hypoxic Preconditioning in CNS

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1. Introduction

The phenomenon that hypoxic preconditioning (HP) protects against subsequent severer anoxia was discovered approximately two decades ago. Subsequently, the effects of HP have been studied intensively in the whole brain, as well as in living hippocampal or cortical slices, and in *in vitro* cell cultures using various hypoxic model systems [1,8,10,12,14,18,28,36,53,56,67,93,94]. Although the exact mechanisms are not completely disclosed, the underlying molecular mechanisms have been postulated. For example, HP activates a great variety of endogenous protective mediators and/or inhibits amounts of harmful mediators, which combined attenuates a burst of free radicals and ultimately increases the capability of cell survival under severe oxygen deprivation [65,74,93,94].

The central nervous system (CNS) is extremely sensitive to a decrease of oxygen content, due to its high intrinsic rate of oxygen consumption [55]. O_2 consumption in brain is maximum in all organs but with a lesser weight ratio. Moreover, the repair ability of CNS is weak to multiple injuries. So, prevention is more important than treatment in CNS. The hypoxic diseases in brain mainly includes stoke, cerebral palsy, etc. Until now, there are no any effective drugs to protect brain from these diseases. Disclosure of the mechanism of HP will contribute to drug discovery for prevention against hypoxic diseases.

A number of cellular adaptive responses to hypoxia are mediated by a key transcription factor termed hypoxia-inducible factor 1 (HIF-1). Activation of HIF-1 by HP enhances the capability to tolerate severe anoxia or ischemia. The target genes of HIF-1, on the one hand, are involved in energy homeostasis, such as erythropoietin (EPO) in the regulation of erythropoiesis [7,29,30,54,71,89], vascular endothelial growth factor (VEGF) in angiogenesis [6,7,88], glucose transmitters (GLUTs) in glucose uptake [62,96] and glycolytic enzymes of anaerobic glycolysis [5,40,80,82], and on the other hand, in redox homeostasis, such as Bcl-2 and adenovirus E1B 19 kDa-interacting protein 3/BNIP3-like (BNIP3/BNIP3L) and microRNA miR-210 in reduction of reactive oxygen species (ROS) [24,43,60,79,97].

ROS are burst from mitochondria during anoxia owing to the lack of O_2 as a final electron acceptor. A mass of ROS are normally regarded as toxic substances due to their strong aggressivity to biological macromolecule, such as proteins, lipids, DNA, and even organelles [52,57]. The macromolecule or organelles damaged by ROS confer oxidative injury, and ultimately result in cell death. In this regard, reduction of ROS during anoxia/ischemia

should lead to the protection of cells. However, a moderate amount of ROS is a stimulus to stabilize HIF-1α [16,69,72,84], a functional subunit of HIF-1, which degrades in normoxia. The duplex roles of ROS will be discussed in this chapter.

During anoxia, a change of cell volume was seldom observed by researchers, however, we noticed the phenomenon and found for the first time that cell volume could be regulated by HP, which caused the protection against severe anoxia. Further analysis showed that sorbitol might mediate the protection [95].

This chapter will review the neuroprotective effects of HP and elaborate the above mentioned mechanisms of HP against anoxia.

2. Activation of HIF-1 by HP and its neuroprotective role in ischemic or anoxic damage

2.1 Characteristic of HIF-1

HIF-1 acts as a pivotal mediator in adaptive responses to hypoxia. It is a heterodimeric transcription factor consisting of an oxygen-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit. Under normoxia, prolyl sites at 402 and 564 residues of HIF-1α are hydroxylated by proline hydroxylases (PHDs, also known as HIF prolyl hydroxylases, HPH and Egg-laying deficient nine, EGLN) utilizing O_2 and α-ketoglutarate as substrates, Fe^{2+} and Vc as cofactors. Hydroxylated HIF-1α is recognized by a tumor suppressor protein Von Hippel Lindau (VHL), an E3 ubiquitin ligase complex, and then HIF-1α is polyubiquitinated by ubiquitins and hence marked for destruction. Ultimately, HIF-1α is degraded in proteasome by hydrolases [17,21,41,42,72,73,91]. In contrast, 803 site of a conserved asparagine residue in HIF-1α is hydroxylated by factor inhibiting HIF-1 (FIH-1), which blocks the binding of the transcriptional co-activator p300/CBP to the site in HIF-1α and leads to the obstruction of transcriptional activation of HIF-1α. Under hypoxia, hydroxylase activity is inhibited due to lack of O_2 as substrate. On the one hand, 402 and 564 sites of prolyl residues cannot be hydroxylated by PHDs, and therefore HIF-1α cannot be degraded through ubiquitin-proteasome pathway. On the other hand, 803 site of asparagine residue cannot be hydroxylated by FIH-1, and hence HIF-1α has the function of transcriptional activation [35,49,59,70,86].

When the stabilized HIF-1α translocates to nucleus, it heterodimerizes with HIF-1β to form a heterodimeric transcription factor complex HIF-1. Then HIF-1 binds to a conserved cis-regulatory motif called the hypoxia-response element (HRE) that contains the core site of 5'-(A/G)CGTG-3' on its target genes. By interaction with coactivators that are required for transcriptional responses to hypoxia, more than two hundred genes are transactivated by HIF-1. HIF-1 target genes include growth and survival factors, such as VEGF, EPO, heme oxygenase 1 (HO-1) [4,63,99], adrenomedullin (AM) [58], inducible nitric oxide synthase (iNOS) [51], etc.; glucose metabolism, such as glucose transporters GLUT1, GLUT3, phosphoglycerate kinase 1, pyruvate kinase M, lactate dehydrogenase A [78,96], etc.; molecules stabilizing homeostasis of redox, such as BNIP3/BNIP3L [79,97], miRNAs [24], etc., which allow cells to adapt to the hypoxic environment.

In addition to stabilizing HIF-1α protein, hypoxia also leads to increased transcription of HIF-1α mRNA and increased expression of HIF-1α protein in brain [82]. Briefly, hypoxic stimuli

play important roles in the accumulation of HIF-1α protein and in transcriptional activation of HIF-1. It has been found that the accumulation of HIF-1α in tissues or cells promoted adaptive mechanisms for cell survival and mediated the tolerance induced by HP [1,87].

2.2 The vital role of HIF-1 in HP

Hypoxic preconditioning (HP) is an exogenous phenomenon in which brief episodes of a hypoxic sublethal insult induces protection against the deleterious effects of subsequent lethal anoxia or ischemia [39,87]. The brain is one of the first organs to fail in hypoxia due to its high intrinsic rate of oxygen consumption. Kitagawa et al. first reported the cerebral HP in 1990 [45]. HP (3 h at 8%O_2) for 24 h before a unilateral occlusion of the common carotid artery in the neonatal rat brain promoted ischemic tolerance [27]. Miller and Bernaudin separately used two different models of hypoxia-induced ischemic tolerance in the adult mouse brain [6,61]. And Bernaudin demonstrated that the tolerance was in association with an increased expression of HIF-1. However, there is no study shown that the effects of hypoxic preconditioning are interrupted by deficiency or blockage of HIF-1 because systemic disruption of the HIF-1 gene leads to embryonic lethality until conditional knockout HIF-1 mice are created. Taie et al. used neural cell-specific HIF-1α-deficient mice to elucidate the role of HIF-1α in hypoxic preconditioning in the brain [87]. . Their results indicated that the protective effects of HP were partially mediated by improving tissue oxygenation via HIF-1α. Similarly, taking advantage of the Cre/Lox technology to generate conditional mutant mice with deletion of HIF-1α predominantly in neurons of the forebrain, Baranova provided evidence that these mutant mice subjected to transient focal cerebral ischemia induced by middle cerebral artery occlusion (MCAO) deteriorated ischemic brain damage. To verify the beneficial role of HIF-1 in the ischemic brain, mice were treated with pharmacologic HIF-1 activators and found that severity of brain ischemic damage was lessened [2]. These researches indicates that activation of HIF-1 by HP is essential to ischemic or anoxic tolerance. However, in contrast to the above results, late-stage brain-specific knock-out of HIF-1α in adult mice reduces rather than increases hypoxic-ischemic damage, and the data suggest that in acute hypoxia, the neuroprotection found in the HIF-1α-deficient mice is mechanistically consistent with a predominant role of HIF-1α as proapoptotic and loss of function leads to neuroprotection [34].

In addition, the stabilization of HIF-1α by HP has been shown to be dose-dependent. Preconditioning of mice dose-dependently stabilized HIF-1α in the retina: exposure to 6 and 10% oxygen for 6 h had the most profound effect on HIF-1α stabilization, while exposure to 14% oxygen stabilized HIF-1α to intermediate levels, and 18 and 21% (normoxia) conditions did not cause a notable stabilization of this transcription factor. Hypoxic HIF-1α stabilization was transient as reoxygenation for 1 h was sufficient to result in the complete degradation of the protein [29].

2.3 The important target genes of HIF-1

Being a transcription factor, HIF-1 plays its important roles via activation of its target genes rather than by itself. The increased expression of HIF-1 by HP results in transcriptional activation of a number of target genes involved in erythropoiesis, angiogenesis, vasodilation, glucose transport, anaerobic glycolysis and autophagy [40,77,79,81,90]. These

target genes are the effectors carrying out the protection from severe anoxia or ischemia, whereas HIF-1 is the sensor or executor of the response to hypoxia. Therefore, activation of HIF-1 and its target genes by HP are equal importance to adaptive mechanisms underlying the prevention against ischemic or anoxic damage. By oligonucleotide microarrays to examine genomic responses in neonatal rat brain following 3 h of hypoxia (8% O_2) and either 0, 6, 18, or 24 h of re-oxygenation, Bernaudin showed that 12 HIF-1 target genes including EPO, VEGF , GLUT-1, AM, etc. were involved in brain hypoxia-induced tolerance. The results suggested that HP-induced HIF-1 target genes might mediate neuroprotection against subsequent ischemia, and might provide novel therapeutic targets for treatment of cerebral ischemia [7].

2.4 EPO

The main role of EPO is the stimulation of erythrocyte production. Studies of human and rat brain show that cerebral EPO is produced by both neuronal and glial cells and that neurons, glia, and cerebral endothelial cells all express the EPO receptor [33]. In mice focal cerebral ischemia model, Leconte et al. demonstrated that a late application of hypoxia 5 days after ischemia reduced delayed thalamic atrophy. Further, with an in vitro oxygen glucose deprivation (OGD) model, they found that HIF-1α and the target gene EPO were both increased by hypoxic postconditioning and revealed that EPO was involved in hypoxia postconditioning-induced neuroprotection [50]. In another report, Bernaudin showed that HP with 8% O_2 of 1-hour, 3-hour, or 6-hour duration for 24 hours before ischemia reduced infarct volume caused by focal permanent ischemia in adult mouse brain by approximately 30% when compared with controls, and they also demonstrated that HP rapidly increased the nuclear content of HIF-1α as well as the mRNA level of EPO and its protein level was up-regulated 24 hours after 6 hours of HP. Therefore, they proposed that HIF-1 target genes contributed to the establishment of ischemic tolerance [6]. A report from Prass et al. demonstrated that HP for 180 or 300 minutes induced relative tolerance to transient focal cerebral ischemia, as evidenced by a reduction of infarct volumes to 75% or 54% of the control, respectively, and they found a marked activation of HIF-1 DNA-binding activity and a 7-fold induction of EPO transcription. Infusion of soluble EPO receptor that neutralizes EPO significantly reduced the protective effect of hypoxic pretreatment by 40%. This study was the first to present functional evidence that EPO is an essential mediator of protection in HP. Accordingly, the authors concluded that endogenously produced EPO is an essential mediator of ischemic preconditioning [71]. Recently it has been reported that HP increased secretion of EPO and up-regulated expression of HIF-1α, B cell lymphoma/lewkmia-2 (Bcl-2), erythropoietin receptor (EPOR), neurofilament (NF), and synaptophysin in ES cell-derived neural progenitor cells (ES-NPCs). Interestingly, HP-primed ES-NPCs survived better 3 days after transplantation into the ischemic brain (30-40% reduction in cell death and caspase-3 activation), and transplanted HP-primed ES-NPCs exhibited extensive neuronal differentiation in the ischemic brain, accelerated and enhanced recovery of sensorimotor function when compared to transplantation of non-HP-treated ES-NPCs [89]. The increased secretion of EPO by HP may play the crucial role in the ischemic brain. It has also been reported that EPO acts at EPO receptors to activate Janus kinase-2 (Jak2), which initiates phosphorylation of inhibitor of NF-κB (IκB) to activate nuclear factor-κB

(NF-κB) and induce NF-κB neuroprotective genes [20]. It is another pathway of EPO in neuroprotection.

2.5 VEGF

VEGF is expressed both in endothelial cells and in neural cells (neurons, astrocytes and microglia). It has the ability to promote cerebral angiogenesis and vasodilation. Induction of VEGF by HP is an attempt to increase tissue oxygen levels by improving blood circulation through the formation of new vessels. Therefore, endogenous VEGF over-expression stimulated by HP should be beneficial following stroke by improving oxygen and nutrient delivery to the ischemic area. In the study of ischemic tolerance, it has been reported that HP with normobaric hypoxia induced ischemic tolerance in adult mice, and increased the nuclear content of HIF-1α as well as the mRNA and protein levels of VEGF [6]. Although the authors did not provide a direct cause-relation effect of VEGF on tolerance induction, the increased expression of VEGF at the time of tolerance appearance provided an indirect argument speaking for the possible implication of HIF-1α and its target genes in this phenomenon. In contrast, the $VEGF^{\partial/\partial}$ knock-in mice, which lack the HRE in the VEGF promoter, reduced hypoxic VEGF expression and caused motoneuron degeneration. This finding suggests an important role for VEGF in neuronal development and maintenance within the central nervous system [48,68].

In the retina, VEGF is also recognized as a pro-survival factor protecting retinal neurons against ischemic injury. Nishijima et al. demonstrated that ischemic preconditioning 24 hours before ischemia-reperfusion injury increased VEGF-A (also called VEGF) levels and substantially decreased the number of apoptotic retinal cells. The protective effect of ischemic preconditioning was reversed after VEGF-A inhibition. Thus, they hypothesized that treatment with VEGF-A might provide neuroprotection in the retina, particularly during ischemic eye disease [64]. Cerebellar granule neurons exposed to 5% O_2 for 9 h showed increased levels of VEGF, VEGF receptor-2 (VEGFR-2), phosphorylated Akt/protein kinase B (PKB), and extracellular signal-regulated kinase 1 (ERK1). Incubation with a neutralizing anti-VEGF antibody, a monoclonal antibody to VEGFR-2, wortmannin, or antisense-Akt/PKB, an ERK-inhibitor, reversed the resistance acquired by HP. Inhibition of VEGFR-2 blocked the activation of Akt/PKB. Pretreatment with recombinant VEGF resulted in a hypoxia-resistant phenotype in the absence of HP. These data indicate a requirement for VEGF/VEGFR-2 activation for neuronal survival mediated by HP and suggest VEGF as a hypoxia-induced neurotrophic factor [92]. The above findings implicate the potential use of VEGF as a therapeutic in neural ischemic or anoxic diseases.

2.6 HO-1

Heme oxygenase (HO) belongs to the heat-shock protein families. It is the rate-limiting enzyme for oxidizing heme to biliverdin and carbon monoxide. Biliverdin is further metabolized to bilirubin, which is a strong antioxidant. By means of guanylyl cyclase, carbon monoxide works as an intracellular messenger, similar to nitric oxide [4]. The isoform heme oxygenase 1 (HO-1; also called HSP32) was found to be inducible in a variety of stress conditions, such as hypoxia [25,47], heat shock [66], hydrogen peroxide [38,55] and so on. The induction is considered to be a cellular adaptive protection due to the antioxidation of HO-1[4,38,55,63,98].

HO-1 is a HIF-1 target gene that has been shown to be expressed in the cerebellum following focal ischemia and in the retina following repetitive HP [33]. Because ischemia or anoxia have been associated with increased ROS, clearance of ROS by production of antioxidants by HO-l could help protect the brain from oxidative injury. As a downstream gene of HIF-1, HO-1 can be induced by HP. Garnier et al. demonstrated that HP induced a progressive and sustained expression of HO-1, and they got a conclusion that antioxidant enzymatic defenses in response to hypoxia might be involved in the protective effect of HP against hypoxia–ischemia [25]. Similarly, in rat liver HO-1 is also involved in the protection exerted by HP against hepatic ischemia-reperfusion (I/R) injury. Lai et al. showed that the levels of HO-1 mRNA and protein were obviously over-expressed after 2 weeks of HP. HP diminished the injury after I/R, while after inhibition of HO-1 activity by zinc protoporphyrin (ZnPP), the protective effect of HP was lessened [47]. Furthermore, they demonstrated that pharmacological preconditioning with simvastatin protected liver from I/R injury by HO-1 induction [46]. In contrast, suppressing HO-1 expression in the presence of HO-1 siRNA during I/R injury, apoptosis was enhanced, whereas HO-1 over-expression attenuated apoptosis [55]. It suggests the anti-apoptosis activity of HO-1.

2.7 BNIP3

BNIP3 (Bcl-2/adenovirus E1B 19-kDa interacting protein 3), BH3-only Bcl-2 family member, localizes to mitochondria when overexpressed [9]. It positively regulates autophagy by competing with beclin-1, a highly conserved protein that is required for the initiation of autophagy, for binding to Bcl-2 or Bcl-X_L, thereby releasing beclin-1 to induce autophagy. Activation of BNIP3-dependent autophagy decreases mitochondrial mass and ROS formation [3,97].

BNIP3 is a known HIF-1 target gene. The promoter has two HIF-1 binding sites. BNIP3 is suppressed by Von Hippel-Lindau (VHL) protein in a renal cell carcinoma cell line, consistent with its regulation through the HIF-1α pathway [85]. In an ischemia-reperfusion model, BNIP3 induced autophagy, which protected myocytes from cell death [31]. Zhang et al. showed that hypoxia induced mitochondrial autophagy and this process required the HIF-1-dependent expression of BNIP3 and the constitutive expression of Beclin-1. The authors proposed a molecular pathway: prolonged hypoxia stimulates HIF-1α activity that up-regulates BNIP3 expression, then over-expression of BNIP3 disrupts the interaction of Beclin-1 with Bcl-2, and as a result, BNIP3 competitively binds to Bcl-2, which leads to Beclin-1 release. Subsequently, Beclin-1 recruits autophagy related proteins that induce the occurrence of mitochondrial autophagy (also called mitophagy). After the defective mitochondria are cleared by mitophagy, cell survival is enhanced under hypoxia due to the reduction of ROS by mitophagy.

In addition, on the basis of the characteristics of HIF-1, pharmacological stabilization or activation of HIF-1α will contribute to the protection against ischemia or severe anoxia, imitating the effect of HP. Desferrioxamine (DFO) and cobalt chloride ($CoCl_2$), an iron chelator and competitive inhibitor of iron, respectively, usually used as a positive control are extensively used agents to mimic the effect of HP by inhibiting PHD enzyme activity and thus stabilize HIF-1α [33,55,78,91]. Additionally, the 2-OG analogues L-mimosine (L-mim), dimethyloxalylglycine (DMOG), and 3,4-dihydroxybenzoate (3,4-DHB) can also be used to inhibit PHD enzyme activity and stabilize HIF-1α [33,71]. Recently it has been

shown that a PHD inhibitor, 2,4-pyridinedicarboxylic acid diethyl ester, pretreatment followed by a 30-min oxygen-glucose deprivation enhanced neuronal resistance in organotypic hippocampal slices on a model of ischemic damage, which was similar to the effect of anoxia preconditioning in the same model system [56]. With the development of novel PHD inhibitors for the treatment of ischemic diseases, a clinical treatment will be promising.

Taken together, these observations suggest that HIF-1 is involved in mediating the beneficial effects of preconditioning, and that pharmacological activation of HIF-1 may be beneficial in stroke and other hypoxic diseases.

3. The duplex roles of ROS

3.1 Formation of ROS

ROS are small and highly reactive molecules that can damage proteins, lipids, DNA and even mitochondria. In mitochondria, respiration generates ROS and ROS are constantly produced during normal metabolism. The balance of ROS formation and removal of ROS via enzymatic activity or antioxidants establishes constitutive ROS levels. ROS include superoxide anions ($O^{2-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$). $O^{2-\bullet}$ are the most common ROS, but can be readily converted to other types by enzymatic (e.g. superoxide dismutase) and non-enzymatic reactions. $O^{2-\bullet}$ are formed by metabolic processes (especially in mitochondria) or by enzymatic reactions (e.g. NADPH oxidase). Multiple isoforms of superoxide dismutase (SOD) can catalyze $O^{2-\bullet}$ to form H_2O_2. $^{\bullet}OH$ is formed from the breakdown of H_2O_2 by transition metals such as iron (Fe^{2+}) or copper (Cu^{+}) through *Fenton reactions*; this is a particularly reactive form of ROS with a short half life. $^{\bullet}OH$, in turn, can form from $O^{2-\bullet}$ and H_2O_2 via the *Haber-Weiss reaction* [100].

Hypoxia and re-oxygenation are capable of stimulating ROS formation in brain tissue. Hypoxia stimulates ROS formation from mitochondria and xanthine oxidase in the cortex, whereas reoxygenation induces NADPH oxidase-derived ROS formation. Although single bouts of hypoxia or re-oxygenation increase ROS formation, repetitive hypoxia/re-oxygenation events amplify this effect. Greater ROS formation during and/or following repeated hypoxia and re-oxygenation may contribute to the pattern-sensitivity of hypoxia-induced respiratory plasticity [57].

3.2 Damage roles of ROS and rescue by autophagy

Excess ROS can damage DNA and proteins, contributing to aging, cardiac disease, cancer and other pathologies [23]. The damage can induce the mitochondrial permeability transition (MPT) caused by opening of non-specific high conductance permeability transition (PT) pores in the mitochondrial inner membrane. ATP depletion from uncoupling of oxidative phosphorylation then promotes necrotic cell death, whereas release of cytochrome c after mitochondrial swelling activates caspases and onset of apoptotic cell death. The defective mitochondria have the potential for futile ATP hydrolysis, which accelerates production of ROS and release of proapoptotic proteins. ROS also attack nucleic acids and are thus genotoxic. A lack of histones in mitochondrial DNA (mtDNA) accounts, at least in part, for a 10- to 20-fold higher mutation rate of mtDNA compared to nuclear

DNA [44]. It is thus clear that elimination of dysfunctional mitochondria is essential to protect cells from the injury of disordered mitochondrial metabolism.

Cells can activate a range of pathways to eliminate ROS, or modulate ROS levels to facilitate essential cellular repair [101]. Mitochondrial autophagy has recently been described as an adaptive metabolic response to prevent increased levels of ROS and cell death [97]. It is now widely accepted that autophagy is crucial for removal of damaged mitochondria by mitophagy. Activation of BNIP3-dependent mitophagy decreases mitochondrial mass and ROS formation, thus supporting cell survival under hypoxia [102]. Removal of dysfunctional mitochondria or oxidized proteins by autophagy is suggested to take place via the chaperone-mediated autophagy (CMA) pathway [103]. By interacting with LC3, p62 mediates the targeting of damaged mitochondria into autophagosomes. More recently, p62 has been implicated in the delivery of oxidized proteins to autophagosomes for degradation. Initially, p62 was thought to act solely through the ubiquitin-proteasome system. However, growing data demonstrates the crucial role of p62 in delivering oxidized protein aggregates to autophagosomes [75]. Removal of damaged mitochondria and oxidized proteins, in most cases, supports survival. Therefore, autophagy is primarily a survival mechanism in response to ROS.

In addition to autophagy, HIF-1 pathway is also involved in decreasing ROS levels. HIF-1 reduces ROS production under hypoxic conditions by multiple mechanisms including: a subunit switch in cytochrome c oxidase from the COX4-1 to COX4-2 regulatory subunit that increases the efficiency of complex IV [104]; induction of pyruvate dehydrogenase kinase 1, which shunts pyruvate away from the mitochondria [105]; and induction of microRNA-210, which blocks expression of the iron-sulfur cluster assembly proteins ISCU1/2 that are required for the function of the tricarboxylic acid (TCA) cycle enzyme aconitase and electron transport chain (ETC) complex I [79]. These HIF-1-mediated mechanisms in maintaining redox homeostasis suggest an adaptive response to hypoxia.

3.3 Beneficial roles of ROS through stabilizing HIF-1

In contrast to the potential damaging effects of ROS, recent studies suggest that generation of mitochondrial ROS, especially H_2O_2, is required for hypoxic HIF-1 activation and stabilization [22,32,54]. In human lung epithelial A549 cells, over-expression of antioxidant enzymes that scavenge H_2O_2, such as catalase or glutathione peroxidase 1 (GPx 1) prevents hypoxic stabilization of the HIF-1α protein. However, over-expression of superoxide dismutase 1 or 2, which detoxifies superoxide to H_2O_2, does not alter hypoxic HIF-1 stabililization [11]. Furthermore, low dose exogenous H_2O_2 could trigger HIF-1α expression and thereby, contribute to hypoxic/ischemic (H/I) preconditioning protection in the immature brain. Chang et al. reported that H_2O_2 induced HIF-1 α protein expression in a dose-dependent manner and provided neuroprotection against severe oxygen-glucose deprivation (OGD) 24 h later. They observed that low dose of exogenous H_2O_2 not only conferred cells a tolerance to subsequent lethal insult, but also alone significantly up-regulated HIF-1α protein expression, suggesting that H_2O_2 produced during OGD preconditioning may stabilize and upregulate HIF-1α. HIF-1α stabilized by endogenous H_2O_2 induced by H/I preconditioning might mediate H/I preconditioning protection [15]. Using superoxide dismutase (SOD1) transgenic (Tg) mice, Liu et al. found that hypoxic preconditioning (HP) was protective in wild-type (Wt) neurons but not in neurons obtained

from SOD1 Tg mice [54]. In Wt neurons, HIF-1α and EPO expression showed a greater increase after hypoxia compared with Tg neurons. Therefore, the authors concluded that HP induced ROS, which might downregulate the threshold for production of HIF-1α and EPO expression during subsequent lethal hypoxia, thus exerted neuroprotection [54].

In conclusion, ROS plays completely opposite roles depending on the amount and the time of ROS production: less ROS and earlier generation has beneficial roles; conversely, excess ROS and later generation has damaging effects.

4. Regulation of cell volume

Cell volume can be regulated by HP to prevent severe anoxic injury. We demonstrated for the first time that HP protected PC12 cells against necrosis after exposure to acute anoxia (AA), and this protective role of HP was probably related to cell volume regulation by increasing aldose reductase (AR) and sorbitol levels [95].

AR is the first enzyme of the polyol pathway. In this pathway, AR catalyzes conversion of glucose to sorbitol in the presence of nicotinamide adenine dinucleotide phosphate, while sorbitol dehydrogenase converts sorbitol to fructose in the presence of NAD^+ [37]. Under osmotic stress, the abundance of AR is elevated by increasing the transcription of its gene. In turn, sorbitol synthesis is raised by increasing the amount and activity of AR [13]. A change of osmolarity causes sorbitol to leak rapidly to the external medium through a sorbitol permease transport pathway, which prevents excessive cell swelling. Efflux of sorbitol was the primary mechanism for regulatory volume decrease (RVD). RVD protects the cells by minimizing swelling [26].

Sorbitol synthesized from glucose catalyzed by AR is directly related to cell volume regulation [19,76,83]. After synthesis, sorbitol is packed into the secretory vesicles to carry and fuse to cytoplasmic membrane, and finally sorbitol is released from it, which is a procedure involving the help of cytoskeleton [19]. Its biological significance is related to cell volume regulation [13,83].

In this study, we showed AA caused a sharp rise in LDH leakage indicative of cell injury, while HP clearly inhibited it. In addition, BB, an inhibitor of AR, completely reversed the protection of HP. These results indicated that AR was involved in the protection produced by HP. AA furthermore caused the increase in cell volume, which would result in swelling and eventually lead cells to necrosis. By observing the change of cell volume at different time points, we found that HP not only delayed the appearance of RVD but also inhibited the increase of cell volume during 24 h of AA exposure. This suggested that cell volume regulation could be a potential mechanism in the protection exerted by HP against AA. We further demonstrated that HP significantly increased sorbitol levels, while the inhibitor of AR, BB, attenuated the increase in sorbitol content induced by HP. According to the above results, we hypothesized that sorbitol might be correlated with increased AR by HP. The fact that quinidine, a stronger inhibitor of sorbitol, reversed the protection afforded by HP indicates that sorbitol contributes to the protection of HP [95].

In summary, HIF-1, ROS and regulation of cell volume may mediate the protection of HP against anoxic or ischemic injury in CNS. Disclosure of the mechanisms of HP will contribute to the prevention and treatment of anoxic or ischemic diseases in brain.

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6. References

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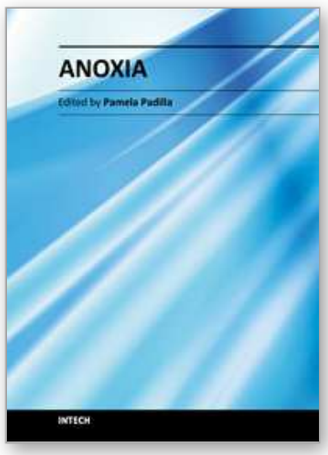

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This book reviews how severe oxygen deprivation affects biological systems - from the molecular to the ecological level. The contributing authors come from diverse regions of the world, which proves the interest in the academic analysis of oxygen deprivation. The diversity in the experimental approach scientists take, in order to understand the influence oxygen deprivation has on living systems, is apparent throughout this book. One of the presented ideas deals with the exploration and examination of the physiological, cellular and genetic characteristics of killifish embryos and nematodes exposed to anoxia. Furthermore, the book includes material on the mechanisms regulating hypoxia and anoxia tolerance and their implications of on human health issues. Finally, new methodologies to examine oxygen deprivation and the impact of human-related activities on oxygen level, within important ecological systems such as Lake Victoria, are presented. There is no doubt that the oxygen molecule is central to every stratum of biological systems.

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